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CONTRACT NO: DAMD17-90-C-0050

TITLE: MOLECULAR STUDIES OF ALPHAVIRUS IMMUNOGENICITY

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CONTRACTING ORGANIZATION: California Institute of Technology  
1201 E. California Boulevard  
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REPORT DATE: April 23, 1992

TYPE OF REPORT: Annual Report

PREPARED FOR: U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND  
Fort Detrick, Frederick, Maryland 21702-5012

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92-13612

92 5 21 070

## REPORT DOCUMENTATION PAGE

1a. REPORT SECURITY CLASSIFICATION <b>Unclassified</b>		1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY		3. DISTRIBUTION/AVAILABILITY OF REPORT <b>Approved for public release; distribution unlimited</b>	
2b. DECLASSIFICATION/DOWNGRADING SCHEDULE			
4. PERFORMING ORGANIZATION REPORT NUMBER(S)		5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION <b>California Institute of Technology</b>		6b. OFFICE SYMBOL (If applicable)	
6c. ADDRESS (City, State, and ZIP Code) <b>1201 E. California Boulevard Pasadena, California 91125</b>		7a. NAME OF MONITORING ORGANIZATION	
7b. ADDRESS (City, State, and ZIP Code)			
8a. NAME OF FUNDING/SPONSORING ORGANIZATION <b>U.S. Army Medical Research &amp; Development Command</b>		8b. OFFICE SYMBOL (If applicable)	
8c. ADDRESS (City, State, and ZIP Code) <b>Fort Detrick Frederick, Maryland 21702-5012</b>		9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER <b>Contract No. DAMD17-90-C-0050</b>	
10. SOURCE OF FUNDING NUMBERS			
		PROGRAM ELEMENT NO. <b>61102A</b>	PROJECT NO. 3M1- <b>61102BS12</b>
		TASK NO. <b>AB</b>	WORK UNIT ACCESSION NO. <b>WUDA346101</b>
11. TITLE (Include Security Classification) <b>MOLECULAR STUDIES OF ALPHAVIRUS IMMUNOGENICITY</b>			
12. PERSONAL AUTHOR(S) <b>James H. Strauss</b>			
13a. TYPE OF REPORT <b>Annual Report</b>		13b. TIME COVERED <b>FROM 3/30/91 TO 3/29/92</b>	
14. DATE OF REPORT (Year, Month, Day) <b>23 April 1992</b>		15. PAGE COUNT	
16. SUPPLEMENTARY NOTATION			
17. COSATI CODES		18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number) <b>Sindbis Virus; Alphavirus; Ockelbo Disease; RA I; BD; Antigenic Epitope; Immunogenicity</b>	
19. ABSTRACT (Continue on reverse if necessary and identify by block number)			
<p>In the past year, our studies on the immunogenicity of alphaviruses have focused on two areas: 1) Determination of the binding site on viral glycoproteins for neutralizing antibodies. Neutralizing antibodies bind to surface structures of a virus and interfere with the uptake or uncoating of the virus. In one case we localized the binding site for a neutralizing monoclonal antibody by using λgt11 expression libraries, following screening of libraries containing random cDNA inserts from Sindbis virus RNA for reactivity with monoclonal antibodies that neutralized the infectivity of Sindbis virus. When combined with sequencing studies of monoclonal escape variants this has allowed us to define an immunogenic domain of alphavirus E2. 2) Sequence analysis of alphaviruses. We are sequencing a number of different strains of Sindbis virus and of its relatives in order to understand the geographic distribution of these viruses and their disease potential. These viruses include Aura virus from South America and Whataroa virus from New Zealand. We have developed cloning methods that have allowed us to obtain cDNA clones representing all of a viral genome which are suitable for high throughput automated DNA sequencing. This has made it possible to acquire sequence data at a much more rapid rate.</p>			
20. DISTRIBUTION/AVAILABILITY OF ABSTRACT <input type="checkbox"/> UNCLASSIFIED/UNLIMITED <input type="checkbox"/> SAME AS RPT. <input type="checkbox"/> DTIC USERS		21. ABSTRACT SECURITY CLASSIFICATION <b>Unclassified</b>	
22a. NAME OF RESPONSIBLE INDIVIDUAL <b>Virginia Miller</b>		22b. TELEPHONE (Include Area Code) <b>301-619-7325</b>	
22c. OFFICE SYMBOL		<b>SGRD-RMT-S</b>	

FOREWORD

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*April 23, 1992*  
DATE



Accession For	
NTIS GRA&I <input checked="" type="checkbox"/>	
DTIC TAB <input type="checkbox"/>	
Unannounced <input type="checkbox"/>	
Justification _____	
By _____	
Distribution/ _____	
Availability Codes	
Dist	Avail and/or Special
A-1	

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## Introduction

The alphaviruses are a widespread group of human pathogens that are present in many parts of the world (Griffin, 1986; Monath, 1988; Peters and Dalrymple, 1990). They are mosquito-borne and are particularly prevalent in tropical and subtropical areas of the world, but alphaviruses pathogenic for man are also present in temperate areas. Many alphaviruses are capable of causing fever, rash and arthralgia in man that in some cases may be disabling for extended periods of time. Many of the New World alphaviruses can cause encephalitis in man. Our program attempts to understand the molecular basis of alphavirus immunogenicity and to determine the relationships of alphaviruses and strains of alphaviruses to one another.

We reported last year that strains of Sindbis virus from Northern Europe referred to as Ockelbo virus and Karielian fever virus, which cause an illness characterized by polyarthritis whose symptoms can persist for months or years, were very closely related to pathogenic strains of Sindbis virus isolated from South Africa (Shirako et al., 1991). We concluded that a South African strain of Sindbis virus was introduced into Northern Europe, probably in the 1960's, either by the activities of man or by migratory birds, and this led to epidemics of Ockelbo disease in Sweden. The virus then spread to Finland and the Karelian region of the Soviet Union, probably in the 1980's, causing epidemics of disease called Pogosta disease and Karelian fever, respectively. We also found that repeated sequence elements found in the 3' nontranslated region of Sindbis viruses are much more highly conserved than sequences outside these elements, and concluded that these repeated elements must play an important role in RNA replication.

In the past year we have continued our sequencing efforts on alphaviruses in order to determine the relations of these viruses to one another. These have included the n<sup>c</sup>P3-nsP4 regions of a South African strain of Sindbis virus in order to examine the relationships within these domains between South African Sindbis viruses and the Northern European Ockelbo viruses

begun last year. We have also examined Whataroa virus, a virus related to Sindbis virus isolated from New Zealand, an Indian isolate of Sindbis virus, and an Australian isolate of Sindbis virus in order to determine the relationships of these viruses to one another. We have begun sequencing of Aura virus in order to search for emergent viruses. We had previously found that Western equine encephalitis virus, found throughout North and South America, arose by recombination between Eastern equine encephalitis virus and a New World alphavirus related to Sindbis virus. Aura virus has been isolated in Brazil and Northern Argentina and is known from serological studies to be related to Sindbis virus. We wished to determine if Aura virus was the second parent of Western equine encephalitis virus.

We are also interested in the localization of neutralizing antibody-binding sites in alphaviruses. The knowledge of immunogenetic domains is important in developing vaccines. Neutralizing antibodies bind to the glycoproteins of alphaviruses and prevent them from attaching to susceptible cells or prevent them from penetrating cells. The exact mechanisms by which neutralizing antibodies inactivate a virus are somewhat controversial and differ from case to case, but at least in some cases the antibody neutralizes by binding to the structure on the surface of the virus that interacts with a receptor on the cell surface, thus directly blocking the virus from interacting with its receptor. In these cases anti-idiotypic antibodies made against such antibodies may function as anti-receptor antibodies. In studies of antibody escape variants we have identified domains of glycoprotein E2 which appear to be important for virus neutralization (Strauss et al., 1991). Here we report that we have been able to use λgt11 expression libraries to directly demonstrate interaction between a neutralizing antibody and a specific domain of glycoprotein E2. Such a result is significant because cases have been described in which resistance to a monoclonal antibody (mAb) arose from single amino acid substitutions away from the actual antibody binding site (Diamond et al., 1985; Parry et al., 1990). Thus it is possible to induce changes in conformation of the antibody-binding regions with amino acid substitutions outside the epitope, and direct demonstration of antibody binding to a defined region is important. Because

this neutralizing monoclonal antibody used here elicits production of anti-idiotypic antibodies which act as anti-receptor antibodies in chicken cells (Wang et al., 1991), this domain is also implicated in attachment to the surface of a susceptible cell. A complete description of these results has appeared in the *Journal of Virology* 65, 7037-7040 (1991). A preprint of this paper entitled "Use of a λgt11 expression library to localize a neutralizing antibody-binding site in glycoprotein E2 of Sindbis virus," by K. S. Wang and J. H. Strauss, was submitted to the U.S. Army Medical Research and Development Command at the time of submission to the journal.

### Methods Used

**Virus Strains.** South African strains of Sindbis virus, Whataroa virus, Indian and Australian isolates of Sindbis virus, and Aura virus were obtained from Dr. J. M. Dalrymple of USAMRIID. Viruses were grown and purified as previously described (Shirako et al., 1991).

cDNA clones for most of the viruses were produced using standard methods (Sambrook et al., 1989). First strand cDNA was made using oligo(dT) as a primer and second strand synthesis was by the method of Gubler and Hoffman (Gubler and Hoffman, 1983). In some cases *Hind*III fragments of the cDNA were cloned into vector pGem3Z. In other cases *Eco*RI linkers were added to double-stranded cDNA and the cDNA cloned into the *Eco*RI site of pGem3Z. DNA sequencing and RNA sequencing used standard technology that is in common use in our laboratory (Hahn et al., 1989; Rice et al., 1985; Rice and Strauss, 1981; Shirako et al., 1991; Strauss et al., 1984).

**Construction of a Random cDNA Library of Virus RNA.** We have also developed methods suitable for high throughput automated DNA sequencing in order to speed up the acquisition of sequence data. For this we used Whataroa virus, strain M78, isolated in 1962 at Westland, New Zealand, from *Culex pervigilans*, as a test virus. The virus was propagated once in primary chicken fibroblast cells and purified by sucrose gradient centrifugation. The RNA was

extracted by an SDS/phenol method, precipitated in ethanol, and suspended in water. First strand cDNA was synthesized with 2 µg of virus RNA using 200 pmol of pd(N)<sub>6</sub> and 2 pmol of dT<sub>17</sub> by AMV reverse transcriptase. The second strand DNA was synthesized by the Gubler and Hoffman (Gubler and Hoffman, 1983) method. The double-stranded cDNA was blunt ended with T4 DNA polymerase in the presence of RNase A, extracted with phenol/chloroform, and precipitated with ethanol. After methylating internal *Eco*RI sites with *Eco*RI methylase, the DNA was electrophoresed in an LMP agarose gel and a 2-4 kb fraction was isolated by a CTAB method as described elsewhere (Shirako and Strauss, 1992). The isolated DNA was kinased with T4 DNA polynucleotide kinase and ligated to kinased *Eco*RI linkers. The ligation products were digested with *Eco*RI, extracted with phenol/chloroform, precipitated with ethanol, and electrophoresed in an LMP agarose gel. The 2-4 kb fraction was isolated by a CTAB method and ligated to an *Eco*RI-digested, CIAP-treated pGEM3Z vector. The ligated DNA was transformed into *E. coli* JM109. One hundred clones that appeared to contain inserts were selected randomly and characterized by restriction analysis of the DNA prepared from 0.5 ml of bacterial cultures. Ninety-six clones were found to contain inserts larger than 1.0 kb. Fifty clones containing larger inserts were further selected and the DNA was prepared from 10 ml of bacterial cultures by a modified boiling method.

**Construction and Screening of the Bacteriophage Library.** Sindbis virus strain AR339, from A. Schmaljohn of USAMRIID, was grown in monolayers of primary chicken embryo fibroblasts (Pierce et al., 1974). Virus was purified as described (Bell et al., 1979), disrupted with 0.5% SDS, and 49S genomic RNA extracted with phenol/chloroform (Hsu et al., 1973). After two ethanol precipitations, RNA was suspended in distilled water and stored at -70°C until use as a template for cDNA synthesis.

A λgt11 library containing short inserts of Sindbis cDNA was constructed by a modification of the procedure of Young and Davis (Young and Davis, 1983). cDNA synthesis

was randomly primed with sonicated salmon testis DNA; [<sup>32</sup>P]dCTP was included during cDNA synthesis in order to monitor the product. After flush-ending with the Klenow fragment of DNA polymerase I, methylation with *Eco*RI methyltransferase, and addition of *Eco*RI linkers (Collaborative Research), the modified cDNA was digested with an excess of *Eco*RI restriction enzyme. The digested cDNA was then fractionated on a Sephadex CL-6B column, and Sindbis cDNA fragments 100-300 base pairs in size were pooled and ligated to dephosphorylated λgt11 arms (Promega). After *in vitro* packaging into phage heads (Stratagene), the percentage of phage containing Sindbis virus cDNA inserts was found to be 90% by plating phage on *E. coli* Y1090 in the presence of 5-bromo-4-chloro-3-indolyl β-D-galactoside. Plaques were screened for reactivity with the various mAbs. Phage plaques were grown for 6 hrs at 42°C, nitrocellulose disks (Schleicher & Schuell) soaked in 10 mM isopropyl thio-β-D-galactopyranoside were then placed on the top of the agar layer, and the plates were transferred to 37°C for 15 hrs. The filters then were lifted and washed successively in 10 mM Tris-Cl pH 7.5 and 150 mM NaCl containing 5% nonfat milk. The filters were incubated overnight at 4°C with monoclonal antibody (10 µg/ml in PBS containing 5% nonfat milk), washed, <sup>125</sup>I-conjugated protein G (0.5 µCi/ml in 5% nonfat milk) added, and the filters were incubated for at least 2 hr at room temperature. After washing and drying, the filters were exposed overnight at -80°C to Kodak-X-OMAT film. Immunoreactive phage were picked and rescreened until a uniformly reactive population was obtained.

#### **Sequence of the nsP3 and nsP4 Region of Alphaviruses**

We have obtained the complete sequence of the nsP3-nsP4 region, approximately 3.5 kb, for four new alphaviruses. These are a South African strain of Sindbis virus isolated from a human case of Sindbis disease, Whataroa virus from New Zealand, an Indian isolate of Sindbis virus, and an Australian isolate of Sindbis virus. Information on these strains and on a number of other strains with which we are currently working, is given in Fig. 1. Shown is the name of the

Subgroup	Strain	Source	Year	Location	Cloning/sequencing status
I	AR339	<i>Culex univittatus</i>	1952	Egypt	Completed (HRsp)
	MP684	<i>Mansonia fuscopennata</i>	1958	Uganda	
	R33	<i>Acrocephalus scriptaceus</i>	1971	Czechoslovakia	
	AR86	<i>Culex sp.</i>	1954	South Africa	50 clones in pGEM
	Girdwood	Human	1963	South Africa	nsP3 nsP4 only
	1038	Turtle dove	1964	Israel	
	Edsbyn82-5 (Ockelbo)	<i>Culiseta sp.</i>	1982	Sweden	Completed
	Edsbyn82-5 (Ockelbo)				
II	A1036	<i>Bdellonyssus ursa</i>	1953	India	nsP3-nsP4 only
	MM2215	<i>Culex tritaeniorhynchus</i>	1955	Indonesia	50 clones in pGEM
	MRM18520	Mosquito pool	1975	Australia	nsP3-nsP4 only
	M78 (Whataroa)	Mosquito pool	1962	New Zealand	nsP3-nsP4 completed
III					

Figure 1 Strains of Sindbis virus used in this study

strain, the source from which the virus was isolated, the year and place of isolation, and the status of our work with the virus.

The four new sequences obtained are presented in Figs. 2 to 5. These nucleotide sequences and the amino acid sequences deduced from them illustrate the close relationships among these alphaviruses and confirm that South African strains of Sindbis virus are very closely related to Ockelbo virus and its allies. nsP4 in particular is very highly conserved. The C-terminal domain of nsP3, which is not highly conserved among alphaviruses, shows more variability, but in each case there is an opal termination codon between nsP3 and the beginning of nsP4 which must be read through in order to produce nsP4.

The relationships among these viruses are illustrated in numerical fashion in Fig. 6. South African Girdwood and Ockelbo exhibit only 1.3% sequence divergence in nsP4 and only 1.8% divergence in the conserved region of nsP3. The Indian and Australian isolates have diverged by 7-10% from these strains in nsP3 and nsP4. Whataroa virus is clearly related to these Sindbis viruses but differs by 12-16% in amino acid sequence in these regions from the Sindbis virus strains.

### High Throughput Automated DNA Sequencing

Several companies, including Applied Biosystems, now make automated DNA sequencers which can greatly speed up the rate of acquisition of sequence data. In order to use such a system, random cDNA clones must be constructed which represent the entire viral genome and DNA must be prepared from such clones that is highly purified and suitable for automated sequencing. We have shown that it is feasible to use the Applied Biosystems sequenator to sequence alphaviruses by using Whateroa virus as a test virus. Random cDNA clones were constructed in a plasmid vector and plasmid DNA was subjected to high throughput automated DNA sequencing. Preparation of plasmid cDNA libraries containing a representative sampling of the Whateroa genome required

Figure 2. nsP3/nsP4 of A1036 (1953, India, *Bdellonyssus bursa*)

1	GCUCGGGCCUAUCGCUAGAACGUGAGAACAUCCGAGUGCCUCGAAGAGGCCGUAGUU A P A Y R S K R E N I A E C L E E A V V	60
61	AAUGC CG GAA UGC ACU CG AC GGG C G A AGGG G U A U G C A A G C C A U A U A A A A A N A A N A L G R P G E G V C K A I Y K K	120
121	UGGCC U A A U A G U U U C G U C G A U U C C G C G A C A G A G A C U G G A A C G G C U A A G C U A G U G U G C U G U W P N S F V D S A T E T G T A K L V C C	180
181	CAAGGAAAGAAAAAUUACCA CG C G U C G G A C C C G A C U U C C G C A A A C A C U C C G A G G C A G A A Q G K K I I H A V G P D F R K H S E A E	240
241	GCACUGAAGAUUCUCCAGAACACAUACCACGCCAUAGCAGAUUUGGUUAACAAACAU G G A A L K I L Q N T Y H A I A D L V N K H G	300
301	AUCAAGACUGUAGCGAUCCCGCUACUAUCCACCGGGAUUUACGCAGCGGGAAAAGACAGA I K T V A I P L L S T G I Y A A G K D R	360
361	CUCGAGGUCCUAAACUGCUUACCGACCGCCCUGGACAGAACAGACGCAGACGUCAC L E V S L N C L T T A L D R T D A D V T	420
421	AUCUACUGCUAGACAAAAAUGGAAAGGAAGGAUCGAUGCGGUUAUACAAUUGAAGGAG I Y C L D K K W K E R I D A V I Q L K E	480
481	UCGGUGACGGAACUGAAGGAUGAGGAUAGGAGAACUGACGAUGAGGUUAUGGUAC S V T E L K D E D M E I D D E L V W I H	540
541	CCGGAUAGUUGUCUCAAGGGCAGGAAGGGUAUAGCACAAACAAAAGGUAAACUUUAUCG P D S C L K G R K G Y S T T K G K L Y S	600
601	UACUUUGAGGGACUAAGUUUCAUCAGGCAGCAAAGACAUGGCGGAGAUUAAAGUACUU Y F E G T K F H Q A A K D M A E I K V L	660
661	UUUCCCGAUGAGCAAGAGUGCAACGAGCAGUUGUGUGCAUACAUCCUUGGUGAAACCAUG F P D E Q E C N E Q L C A Y I L G E T M	720
721	GAAGCCAUCAGGGAAAAAUCCAGUGGACUUUAUCCGUCGUCCAGUCCGCCAAGAC E A I R E K C P V D F N P S S S P P K T	780
781	CUCCCCUGUUUGUGCAUGUAUGCCAUAGCACGCCUGAGAGAGUGGCACCGUCUGCGUAGCAAC L P C L C M Y A M T P E R V H R L R S N	840
841	AACGUCAAGUCCAUCAUCAGUGUGUUCGUACCCACUUCCGAAGCACAAGAUCAAGAAC N V K S I T V C S S T P L P K H K I K N	900
901	GUUCAGAAAGUACAGUGCACGAAAGUGGUUCUUGUCAAUCCACAGACCCUGAAUUGUC V Q K V Q C T K V V L F N P Q T P E F V	960
961	CCUGCCCGUAAGUACAUAGAACGACAACAAAAGACGUAGGCCAAGAUGCAGAAGAAC P A R K Y I E A Q P K D V S Q D A E E S	1020
1021	CCUGCCCGAGCCGCCGAGAUAAACCCUCACGGGACGUACAGACAUUAUCCUGGAUGUG P A A A A R D N T S R D V T D I S L D V	1080
1081	GAAGAAAGUCAAGCCGCAGCCGGCAACCAGAGGAGCGCUCGGGGACAACACUCCCG E E S Q A A A G Q P E E R S G D N T S R	1140
1141	GAUGUAACAGAUUAUCCUAGAUCACGACAGCGAUAGUGAGGAGGGCUCCAUCUUCU D V T D I S L D H D S D S E V G S I F S	1200
1201	AACCUUAGCUGCUCCAGUCAAUCCAUCAUCAGUAUGGACAGCUGGUCCUCCGGACCGGG N L S C S S Q S I T S M D S W S S G P G	1260

Figure 2. nsP3/nsP4 of A1036 (1953, India, *Bdellonyssus bursa*)

1261	UCGAUCACGAUAACGAGAACCGCACCAUUCAGGUACACGGCGGAGAUACACAAUGCUCU S I T I N E N R T I Q V T A E I H N A P	1320
1321	GCCGCGUJGCCUGUJCCACCACCGCCUUAAGAAACUGGCACGCUAGCAGCCCAGAAG A A L P V P P P R L K K L A R L A A Q K	1380
1381	CCCAAUCGCCAUCCGACCCGCCUUCGACGGUCGAGGACGUGUCGAUGCGCUUGGUCCU P N P P S D P P S T V E D V S M R L S F	1440
1441	CCUGCCACGGUGUCGUUCGGAUCAUUCUCCGACGGAGAACUGACGACCUUAGCCGCGAU P A T V S F G S F S D G E V D D L S R D	1500
1501	AAAGCAGUGUCAGAACCGGUUGGUUUUGGUUCUUCGAGCCUGGAGAGGUACCUUAUC K A V S E P V V F G A F E P G E V T S I	1560
1561	AUCGAAUCAAGGUUCUGUGUCAUUCCCGUGCAUAAACGCCGGCGCAGAACGGGG I E S R S V V S F P V H K R R R R R R G	1620
1621	AAAAGAACCGAAUUAUUGACUAACCGGGGUAGGUGGGUACAUUCUCAACUGACACGGGA K R T E Y * L T G V G G Y I F S T D T G	1680
1681	CCGGGCCACCUCCAGAACGAGAACUGAGUUCUGCAAACAGCUACUGAACCGACCCUCGAG P G H L Q K K S V L Q N Q L T E P T L E	1740
1741	CGCAAUCAAUUAGAACGAAUGUAUGCGCCAGUCUCGAUGUCAAGAAAGAGGAACUUCUG R N Q L E R M Y A P S L D V K K E E L L	1800
1801	AAACUUAAGUACCAAAUAGAUGCCCACCGAAGCCAUAAGUAGGUACCAACGUAGAAAG K L K Y Q M M P T E A N K S R Y Q S R K	1860
1861	GUUGAAAAUCAAAAAGCGGUACCCACCGAGAGGUACUGUCGGACUGAAGAUGUACAUC V E N Q K A V T T E R L L S G L K M Y I	1920
1921	CACUCAGAGAACCAACCUGAGUGUUAAGGUACUUAUCCGAAACCGUCGUACUCCAGC H S E N Q P E C Y K V T Y P K P S Y S S	1980
1981	AGUGUCCCCUUAAGUACCAAGAACCCUGAAUUCGCCGUAGCUGUUUGCAAUAACUACCUG S V P L S Y Q N P E F A V A V C N N Y L	2040
2041	CAUGAGAACUACCCGACGGUUGCCUCCUAUCAGAUUACGGACGAUAUGAUGCCUACCU H E N Y P T V A S Y Q I T D E Y D A Y L	2100
2101	GACAUGGUGGACGGCACUGUJUGCGUGUCUCGACACUGCAACAUUCUGCCUGCGAAAUJA D M V D G T V A C L D T A T F C P A K I	2160
2161	CGUAGCUUUCGAAAGAAACAUAGAGUACCGCGCACCACAUACAGGAGUGCCGUGCCGUCU R S F P K K H E Y R A P N I R S A V P S	2220
2221	GCUAUGCAGAACACUCUACAGAACGUCCUGAAUGCAGCAACAAAGAGGAUUGCAACGUU A M Q N T L Q N V L N A A T K R N C N V	2280
2281	ACUCAGAUGAGAGAACUACCGACCCUAGACUCCCGACCUUUAACGUGGAAUGCUCCGA T Q M R E L P T L D S A T F N V E C F R	2340
2341	AAGUACCGCGUGCAAUGACGAGUAUUGCGUGAAUUCGCCGAAAAACAAUCAGGAUCACC K Y A C N D E Y W A E F S E K P I R I T	2400
2401	ACGGAGUUUGUUACGGCGUACGGUGGCAGAUUGAAGGGACCAAAGGCUGCUGCUCUGUU T E F V T A Y V A R L K G P K A A A L F	2460
2461	GCAAAACGCAUAACCUAGUCCCAUUGCAAGAACGUACCUAUGGACAGGUUUGUGAUGGAC A K T H N L V P L Q E V P M D R F V M D	2520

Figure 2. nsP3/nsP4 of A1036 (1953, India, *Bdellonyssus bursa*)

2521	AUGAAGCGAGAUGUCAAGGUGACUCCGGGCACAAAACACACCGAAGAAAGGCCUAAGGUG M K R D V K V T P G T K H T E E R P K V	2580
2581	CACGUAAUCCAAGCGGCUAGCCUUUUGCACAGCCUACCUUUGUGGCAUCCACCGAGAG Q V I Q A A E P F A T A Y L C G I H R E	2640
2641	CUGGUACGCCGGCUUACCGCGGUUCUACUCCGAACGUACACACCCUGUUUGACAUGUCU L V R R L T A V L L P N V H T L F D M S	2700
2701	GCGGAGGAUUUCGACCGCAUUUUGCCGAGCAUUUCCGACAAGGUGACGCCGUGCUCGAG A E D F D A I I A E H F R Q G D A V L E	2760
2761	ACAGACAUUCGCGUCAUUCGAAUAGAGUCAGGACGAUGCGAUGGCCUGACUGGGCUGAUG T D I A S F D K S Q D D A M A L T G L M	2820
2821	AUCCUGGAGGACCUCGGCGUCGAUCAACCGCUGCUGGACCUCAUCGAGUGUGGCCUUCGGA I L E D L G V D Q P L L D L I E C A F G	2880
2881	GAAAUAUCAUCUACGCAUCUGCCUACUGGGACACGGUUUAAGUUCGGCUCAAUGAUGAAA E I S S T H L P T G T R F K F G S M M K	2940
2941	UCCGGAAUGUUUCUUACGCUCUUCGUGAACACCAUCUUGAAUGUCGUGAUUCGUAGUCGC S G M F L T L F V N T I L N V V I A S R	3000
3001	GUGCUUGAGCACAGGUAAACAGGAUCACGAUGUGGCCGCAUUCAUUGGAGACGAUAAACAU V L E H R L T G S R C A A F I G D D D N I	3060
3061	AUCCACGGCGUGGUAAUCAGACAAGGAAUAGGCCGAAAGGUGCGCCACUUGGCUGAAUAUG I H G V V S D K E M A E R C A T W L N M	3120
3121	GAGGUAAAAAUCAUUGACGCGGUGAUCCGGCAGCGGUCCUCCGUAAUUCUGUGGUGGCCUU E V K I I D A V I G E R P P Y F C G G F	3180
3181	AUACUACAGGACUCUGUCACCCAAACAGCCUGUCGAGUGGCUGACCCCCUAAAAGACUG I L Q D S V T Q T A C R V A D P L K R L	3240
3241	UUCAAGCUAGGAAAACCUUUGCCCGAGAUGAUGACCAAGAUGAAGACAGAAGAAGGGCU F K L G K P L P A D D D Q D E D R R R A	3300
3301	UUGCUGGAUGAGACUAAGCGUGGUUAGAGUGGGCAUAACGAAACAUUGGCUACUGCG L L D E T K A W F R V G I T E T L A T A	3360
3361	GUAGCAACGCGGUACGAAGUUGAUACAUACGCCUGUCCUGCUGGCACUGAGGGACCCUU V A T R Y E V D N I T P V L L A L R T L	3420
3421	GCGCAAAGCAAGAGAUCCUUCAGUCCAUAGAGGGAAAUGAAGCAUCUCUACGGUGGU A Q S K R S F Q S I R G E M K H L Y G G	3480
3481	CCUAAAUG 3489 P K *	

Figure 2. Translated sequence of the nsP3-nsP4 region of Sindbis strain A1036, using the single letter amino acid code. nsP3 and nsP4 are translated as part of a polyprotein encoded by nucleotides (nts) 4100 to 7600 in the type virus genome. In this and the following 3 figures, nts are numbered from the amino terminus of nsP3. The star at nt 1636 indicates the opal codon separating nsP3 and nsP4; the star at nt 3489 is the termination codon of nsP4. The amino terminal residue of processed nsP4 is Tyr (Y) encoded by nts 1657-1659.

Figure 3. nsP3/nsP4 of MRM18520 (1975, Australia, mosquito pool)

1	GCUCCGGCCUACCGCUCGAAACGUGAGAAUCGCCGAAUGCCTUUGAAGAGGCCGUAGUU	60
	A P A Y R S K R E N I A E C L E E A V V	
61	AACGCCGCAACCCACUCGGACGUCCGGCGAAGGGGUGUGUAAAGCCAUUAUAAAAAA	120
	N A A N P L G R P G E G V C K A I Y K K	
121	UGGCCCAAUAGUUUUGUCGAUUCUGCGACAGAGACUGGAACAGCUAGCUAGUGUGCUGU	180
	W P N S F V D S A T E T G T A K L V C C	
181	CAAGGAAAAAGAUUAUCCAUUGCCGUCCGACCUGACUCCGUAAAACACCCCGAGGCAGAA	240
	Q G K K I I H A V G P D F R K H P E A E	
241	GCGCUGAAGAUUCUCCAGAACACAUACCACGCCAUUGCAGAUUUGGUAAACAAACAUGGA	300
	A L K I L Q N T Y H A I A D L V N K H G	
301	AUCAAGACCGUAGCGAUCCCGCUUCAUACCACCGGAAUACGCAGCGGGAAAAGACAGA	360
	I K T V A I P L L S T G I Y A A A G K D R	
361	CUUGAGGUCUUUAAAACUGCCUCACUACCGCCUGGACAGAACUGACGCGAGACGUACAC	420
	L E V S L N C L T T A L D R T D A D V T	
421	AUCUACUGCCUUGACAAAAAUGGAAAGAACGGAUUGAUGCUGUUUAACAGUUGAAGGAG	480
	I Y C L D K K W K E R I D A F I Q L K E	
481	UCGGUGACGGAACUGAAGGAUGAUGACAUGGAGAACUGACGACGAAUAGUAUGGAUCCAC	540
	S V T E L K D D D M E I D D D E L V W I H	
541	CCGGAUAGUUGCCUCAAGGGUAGGAAAGGGUUUAGUACGACGAAAGGCAAGCUCUACUCG	600
	P D S C L K G R K G F S T T K G K L Y S	
601	UACUUUGAGGGGACUAAAUUCAUCAAGCAGCAAAGACAUUGGCUGAGAGAUCAAGGUACUU	660
	Y F E G T K F H Q A A K D M A E I K V L	
661	UUUCCCGAUGAGCAAGAGUGCAACGAGCACUGUGUGCAUACAUUCUAGGCAGAACCAUG	720
	F P D E Q E C N E Q L C A Y I L G E T M	
721	GAAGCCAUCAGGGAAAAAUGGUCCAGUGGACUUAAAUCGUCCGUCCAGUCCGCCAGACG	780
	E A I R E K C P V D F N P S S S P P K T	
781	CUUCCCUGUUUGUGUAUGUACGCCAUGACGCCGAGAGAGUGGCACCGCUUGCGUAGCAAU	840
	L P C L C M Y A M T P E R V H R L R S N	
841	AACGUAAAUCCAUCACAGUAUGCUCGUCAACCCCGCUUCCGAAGCACA AAA UUAAGAAC	900
	N V K S I T V C S S T P L P K H K I K N	
901	GUUCAGAAAGUACAGUGCACGAAAGUAGUCCUAUUCAACCCACAAACGCCUGAAUUGUC	960
	V Q K V Q C T K V V L F N P Q T P E F V	
961	CCUGCCCGCAAGUACAUAGAAACACAACCGAAGGACGACAGUCAAGAGGCCAGAAC	1020
	P A R K Y I E T Q P K D D S Q E A E E N	
1021	CCUGCCCGAGCCGAUAACACUUCACGGGAUGUAACAGACGUACUUCUAGAUGUGGAAGGA	1080
	P A A A D N T S R D V T D V S L D V E G	
1081	GAUCGCCGUUGCGGCCAACCGAACAGAGGGCACUCAGAGGACAACACCUCCCGAGAUGUA	1140
	D R V A A N R S E V H S E D N T S R D V	
1141	ACAGACAUAAUCUAGACCACAACAGUGAUAGCGAGGUGGGCUCCAUUUUCUCUGACCUC	1200
	T D I S L D H N S D S E V G S I F S D L	
1201	AGCUGCUCCAGUCAUCCAUCACCAGCAUGGACAGCUGGUCCUCCGGACCGAGCUCGAUC	1260
	S C S S H S I T S M D S W S S G P S S I	

Figure 3. nsP3/nsP4 of MRM18520 (1975, Australia, mosquito pool)

1261	AUGCUAAACGGGAUCACACCACCUCCAGGUACCGGAGAGAUACACAACGCUCUGCUGCA M L N G N H T I Q V T A E I H N A P A A	1320
1321	CCGCCCGUACCACCAACGCCCUAAGAAACUGGCAGCGCUUGGCAGCUCAGAAGUCCGAU P P V P P P R L K K L A R L A A Q K S D	1380
1381	CCGCCAUCCAGCCGCCCUAACGGUUGAGGACGUGUCGAUGCGCCUGUCAUUCCCUGCC P P S S P P S T V E D V S M R L S F P A	1440
1441	ACGGUGUCAUUCGGAUCUUUUUCUGACGGCGAAGUGCACGAUCUUAGUCGCAGAAAGCA T V S F G S F S D G E V D D L S R E K A	1500
1501	GUGUCAGAACAGUGGUUUUGGUCCUUUCGAGCCAGGAGAGGUACAUCAUCAJUGAA V S E P V V F G A F E P G E V T S I I E	1560
1561	GCAAGGUCUGUCGUCAUCCCCGUGAAUAAAAGCCGGCGCAGGAGACGGGGCCAAAAG A R S V V S F P V N K R R R R R R G Q K	1620
1621	AAAACGAAUUAUUGACUAACCGGGGUAGGGGUUAUACUUUCUGACUGACACGGGACCG K T E Y * L T G V G G Y I F S T D T G P	1680
1681	GGUCACCUCCAGAAAAAUCCGUUCUACAAAACCAGCUACGGAACCGAACCCUCGAGCGU G H L Q K K S V L Q N Q L T E P T L E R	1740
1741	AAUCAAUUAGAACGGAGUGUAUGCACCCAGUCUUGAUGCCAAGAAAGAGGAACCUJUGAA N Q L E R V Y A P S L D A K K E E L L K	1800
1801	CUCAGUACCAAAUGAUGGCCACCGAACGCCAAUAAAAGUAGGUACCGACUAGAAAGGU L K Y Q M M P T E A N K S R Y Q S R K V	1860
1861	GAAAACCAAAAGCCGUACCCACCGAGAGGGUACUGUCGGGUAGAGAUGUACAUUCAC E N Q K A V T T E R L L S G L K M Y I H	1920
1921	UCAGAGAACCAACCCGAGUGUUACAAGGUCACCUAUCGAAACCGUCGUACCUAGCAGU S E N Q P E C Y K V T Y P K P S Y S S S	1980
1981	GUUCCCUUAGUACCAAGAGCCCCGAAUUCGCCGUAGCCGUCUGCAAUAACUACCUCAU V P L S Y Q S P E F A V A V C N N Y L H	2040
2041	GAGAAUUAUCCAACGGUUGCCUCCUAUCAGAAUACGGGAUGAAUAGACGCCUACCUUGAC E N Y P T V A S Y Q I T D E Y D A Y L D	2100
2101	AUGGUGGACGGCACCGUAGCGUGUCUGACACCCGUACAUUUJGCCCCGCGAAAUJACGC M V D G T V A C L D T A T F C P A K L R	2160
2161	AGCUUCCGAAGAAACACGAGGUACCGAGAACCUAACAUCAAGGAGCGCCGUACCGUCGC S F P K K H E Y R E P N I R S A V P S A	2220
2221	AUGCAGAACACCUACAGAACGUCCUGAACGCAGCAACAAGAGGAUJGCAAUGUUACU M Q N T L Q N V L N A A T K R N C N V T	2280
2281	CAGAUGAGAGAACUACCGACUUUAGACUCCGCAACCUUUAAUGUGGAAUJGUUCUGAAAG Q M R E L P T L D S A T F N V E C F R K	2340
2341	UACGCGUGCAACGACGAGUAUUGGCUGAAUUCUCGGAAAAACCAAUUAGGAUCACCAC Y A C N D E Y W A E F S E K P I R I T T	2400
2401	GAGUUUGUCACGGGUACGGCGAGAUUGAAGGGACCAAGGCUGCUGCACUGUUJGCU E F V T A Y V A R L K G P K A A A L F A	2460
2461	AAAACGCAUAACCUAGUCCACUGCAAGAAGUACCUAUGGACAGGUUUGUGAUGGACAUG K T H N L V P L Q E V P M D R F V M D M	2520

Figure 3. nsP3/nsP4 of MRM18520 (1975, Australia, mosquito pool)

2521	AAGCGAGACGUUAAGGUGACUCCGGGCACGAAGCACCCGAAGAAAAGACCCAAAGUGCAG K R D V K V T P G T K H T E E R P K V Q	2580
2581	GUAAUCCAAGCGGCAGGCCUCUAGCUACAGCCUAUUUAUGCGGCAUCCACCGUGAGCUG V I Q A A E P L A T A Y L C G I H R E L	2640
2641	GUACGCAGGCUUACCGCAGGUCCUGCUUCCGAACGUACACACCCUUUUUGAU AUGUCUGCG V R R L T A V L L P N V H T L F D M S A	2700
2701	GAAGAUUUCGAUGCUAUCAUUGCCGAGCAUUUCACCAGGGUGACGCUGUGCUCGAGACA E D F D A I I A E H F H Q G D A V L E T	2760
2761	GACAUCCGUCGUUCGAUAAGAGCCAAGACGAUGCGAUGGCCUGACGGGGCUGAUGAUC D I A S F D K S Q D D A M A L T G L M I	2820
2821	CUGGAGGACCUCGGAGUCGACCAGCAUUGCUGGACCUAUCGAGUGCGCCUUCGGGAA L E D L G V D Q P L L D L I E C A F G E	2880
2881	AUAUCAUCUACGCACCUGCCGACCGGGACACGGUUUAAGUUCGGCUCAAUGAUGAAAUC I S S T H L P T G T R F K F G S M M K S	2940
2941	GGAAUGUUCUCACCGCUCUUJUGUGAACACCAUCUUGAAUGUCGUGAUAGCUAGUCGGCUG G M F L T L F V N T I L N V V I A S R V	3000
3001	CUCGAGCACAGGUAGCAGAAUCACGAUGCGCCCAUUCAUUCGGAGACGACAUAUUAUU L E H R L A E S R C A A F I G D D N I I	3060
3061	CACGGCGUGGUAUCCGACAAAGAAAUGGCUGAAAGGGCGCACUUGGCUGAAUAUGGAG H G V V S D K E M A E R C A T W L N M E	3120
3121	GUAAAAAUUUAUGACGCAGUAUUGCGAACGUUCUCCGUACUUCUGUGGGCUUUUA V K I I D A V I G E R P P Y F C G G F I	3180
3181	CUGCAGGACUCAGUCACCCAAACAGCCUGCCGAGUGGGCGGACCCCCUAAAAGAUUGU L Q D S V T Q T A C R V A D P L K R L F	3240
3241	AAAUAAGGAAAACCAUUACCUGCAGAUGAUGACCAAGAUGAAGACAGAAGAAGGGCUCUG K L G K P L P A D D D Q D E D R R R A L	3300
3301	CUGGAUGAGACCAAGGCUGGUUUAGAGUGGGCAUAACUGAGACACUGGCACUGCGGU L D E T K A W F R V G I T E T L A T A V	3360
3361	GCAACCGGGUAUGAAGUUGAUACAUCACACCGGUCCUGCUGGCACUGAGGGACCCUUGCG A T R Y E V D N I T P V L L A L R T L A	3420
3421	CAAAGCAAGAGAUCUUUUCAGGCCAUAGGGAAAUGAAGCAUCUCUACGGUGGUCC Q S K R S F Q A I R G K M K H L Y G G P	3480
3481	AAAUG 3486 K *	

Figure 3. Translated sequence of the nsP3-nsP4 region of the MRM18520 strain of Sindbis from Australia. Conventions are the same as in Figure 2.

Figure 4. nsP3/nsP4 of Girdwood (1963, South Africa, human)

1	GCACCGUCAUACCGCACUA	AAAGGGAGAACAUUGCUGAUUGUCAAGAGGAAGCAGUUGUC	60
	A P S Y R T K R E N I A D C Q E E A V V		
61	AAUGCAGCCAAUCGCUGGGCAGACCAGGCGAAGGGAGUCUGGCCGUGCCAUCUA	AAACGUNA A A N P L G R P G E G V C R A I Y K R	120
121	UGGCCGAACAGUUUCACCGAUUCAGCCACAGAGACCGGCACCGCAAAACUGACUGUGUC	W P N S F T D S A T E T G T A K L T V C	180
181	CAAGGAAAGAAAGUGAUCCA	CGCGGUUGGCCUGAUUUCCGGAAACACCCAGAGGCAGAA	240
	Q G K K V I H A V G P D F R K H P E A E		
241	GCCCUGAAA	UUGCUGCAAACGCCUACCAUCGAGACUAGUAAAUGAACAUAAU	300
	A L K L L Q N A Y H A V A D L V N E H N		
301	AUCAAGUCUGUCGCCAUCCCACUGCUAUCUACAGGCAUUUA	CGCAGCCGGAAAAGACCGC	360
	I K S V A I P L L S T G I Y A A A G K D R		
361	CUUGAAGUAUCACUUA	CUGCUUGACAACCGCGCUAGAUAGAACUGAUGCGGACGUAA	420
	L E V S L N C L T T A L D R T D A D V T		
421	AUCUACUGCCUGGAUAA	AGAAGUGGAAGGAAGAACUGACGCGGUGCUCCAACUUAAGGAG	480
	I Y C L D K K W K E R I D A V L Q L K E		
481	UCUGUAACAGAGCUGAAGGA	AGGAGAUAGGAGAUAGGAGAUACGACGAGAGUAGUAUGGAUCCAU	540
	S V T E L K D E D M E I D D E L V W I H		
541	CCGGACAGUUGCCUGAAGGGAAAGAAAGGGAUUCAGUACUACAAAAGGAAAGUUGUAUUCG	P D S C L K G R K G F S T T K G K L Y S	600
601	UACUUUGAAGGCACCA	AAUUCAUCAACGAGCAAAAGAUAUAGGCGGAGAUAAAGGUCCUG	660
	Y F E G T K F H Q A A K D M A E I K V L		
661	UUCCCCAAUAGACCAGGAAAGCAACGAGCAACUGUGUGCCUACAUAU	UUGGGGAGACCAUGF P N D Q E S N E Q L C A Y I L G E T M	720
721	GAAGCAAUCGCCAAAAA	UUGCCGGUCGACCAACACCGUCGUAGCCGCCAAAAACGE A I R E K C P V D H N P S S S P P K T	780
781	CUGCCGUGCCUCUGCAUGUA	UGGCCAGAAAGGGGUCCACAGACUCAGAAC	840
	L P C L C M Y A M T P E R V H R L R S N		
841	AACGUCAAAGAAGUUA	CAGUAUGCUCCUCCACCCCCCUUCCAAAGUACAAAUAAGAAC	900
	N V K E V T V C S S T P L P K Y K I K N		
901	GUUCAGAAGGUUCAGUGCA	AAAAGUAGUAGGUUUACCCGCAUACCCUGCAUUCGUUV	960
	V Q K V Q C T K V V L F N P H T P A F V		
961	CCCGCCCCGUAA	GUACAUAGAACGCCAGAACAGCCUGCAGCUCCGCCUGCACAGGCCGAGP A R K Y I E A P E Q P A A P P A Q A E	1020
1021	GAGGCC	CCGAAGUUGCAGCAACACCAACACCACCCUGCAGCUGUAACACCU	1080
	E A P E V A A T P T P P A A D N T S L D	CGUUGA U	
1081	GUCACGGAC	AUCUCACUGGACAUGGAAGACAGUAGCGAAGGCCUACUCU	1140
	V T D I S L D M E D S S E G S L F S S F	UUUCGAGCUUU	
1141	AGCGGAUCGGACAAC	CUCUAUUACUAGUAUGGACAGUUGGUCCUGCAGGACCUAGUUCACUA	1200
	S G S D N S I T S M D S W S S G P S S L		
1201	GAGAUAGUAGACCGAAGGCAGG	GGUGGUCCUGACGUCCAUGCCGUCCAGAGCCUGCC	1260

Figure 4. nsP3/nsP4 of Girdwood (1963, South Africa, human)

	E I V D R R Q V V V A D V H A V Q E P A	
1261	CCUGUUCCACCGCAAGGCUAAAGAAGAUGGCCGCCUGGCAGCGGCAAGAAUGCAGGA P V P P P R L K K M A R L A A A R M Q E	1320
1321	GAGCCAACCUCCACCGCAAGCACCAGCUCUGCGGACGAGUCCCUCACCUUUCUUUUGGU E P T P P A S T S S A D E S L H L S F G	1380
1381	GGGGUAUCCAUGGUCCUUCGGAUCCCUUUUUCACGGAGAGAUGGCCCGCUUGGCAGCGGCA G V S M S F G S L F D G E M A R L A A A	1440
1441	CAACCCCCGGCAAGUACAUGCCCUACGGAUGUGCCUAUGCUUUCGGAUCGUUUUCGAC Q P P A S T C P T D V P M S F G S F S D	1500
1501	GGAGAGAUUGAGGAGCUGAGCCGAGAGUAACCGAGUCUGAGGCCGUCCUGUUUGGUCA G E I E E L S R R V T E S E P V L F G S	1560
1561	UUUGAACCGGGCGAAGUGAACUCAUUUAUCGUCCGAUCAGCCGUUAUCUUUCCACCA F E P G E V N S I I S S R S A V S F P P	1620
1621	CGCAAGCAGAGACGUAGACGCAGGAGCAGGAGGACCGAAUACUGACUAACCGGGUAGGU R K Q R R R R S R R T E Y * L T G V G	1680
1681	GGGUACAUAUUUUCGACGGACACAGGCCUUGGCACUUGCAAAGAAGUCCGUUCUGCAG G Y I F S T D T G P G H L Q K K S V L Q	1740
1741	AACCAGCUUACAGAACCGACCUUGGAGCGCAAUGUUUCUGGAAAGAAUCUACGCCCGUG N Q L T E P T L E R N V L E R I Y A P V	1800
1801	CUCGACACGUCGAAAAGAGGAACAGCUAAACUCAGGUACCAGAUGAUGGCCACCGAAGCC L D T S K E E Q L K L R Y Q M M P T E A	1860
1861	AACAAAAGCAGGUACCAGCUAGAAAAGUAGAAAUCAGAAAGCCAUACCACUGAGCGA N K S R Y Q S R K V E N Q K A I T T E R	1920
1921	CUGCUUUCAGGGCUACGACUGUAUAACUCUGCCACAGAUCCAGAAUGCUUAAGAU L L S G L R L Y N S A T D Q P E C Y K I	1980
1981	ACCUACCCGAAACCAUCGUAUUCCAGCAGUGUACCGCGAACUACUCUGACCCAAAGUU T Y P K P S Y S S S V P A N Y S D P K F	2040
2041	GCUGUAGCUGUUUGCAACAACUACUGCAUGAGAAUUCGCCAGGGACAGGUAGCAUCUUACAG A V A V C N N Y L H E N Y P T V A S Y Q	2100
2101	AUCACCGACGAGUACGAUGCUUACUUGGUAAUGGUAGACGGGACAGUCGUUGCUAGAU I T D E Y D A Y L D M V D G T V A C L D	2160
2161	ACUGCAACUUUUUGCCCGCCAAGCUUAGAAGUACCCGAAAGACACGAGGUAGAGCC T A T F C P A K L R S Y P K R H E Y R A	2220
2221	CCAAACAUCCGCAGUGCGGUUCCAUCAGCGAUGCAGAACACGUUGCAAACGUGCUAU P N I R S A V P S A M Q N T L Q N V L I	2280
2281	GCCGCGACUAAAAGAAACUGCAACGUACACAAAUGCUGAAUUGCCAACACUGGACUC A A T K R N C N V T Q M R E L P T L D S	2340
2341	GCGACAUCAACGUUGAAUGCUCUUCGAAAAAUGCAGUAAUGACGAGGUAUUGGGAGGAG A T F N V E C F R K Y A C N D E Y W E E	2400
2401	UUUGCCGAAAGCCAACUJAGGAUCACUACUGAGUUCGUUACCGCAUACGUGGCCAGACUG F A R K P I R I T T E F V T A Y V A R L	2460
2461	AAAGGCCCUAAGGCCGCCACUGUUCGCAAAGACGCAUAAAUGGUCCCAUUGCAAGAA A A T K R N C N V T Q M R E L P T L D S	2520

Figure 4. nsP3/nsP4 of Girdwood (1963, South Africa, human)

	K G P K A A A L F A K T H N L V P L Q E	
2521	GUGCCUAUGGAUAGGUUCGUCAUGGACAUGAAAAGAGACGUGAAAGUUACACCUGGCACG	2580
	V P M D R F V M D M K R D V K V T P G T	
2581	AAACACACAGAAGAAAAGACCGAAAGUACAAGUGAUACAAGCCGCAGAACCCCUGGCGACC	2640
	K H T E E R P K V Q V I Q A A E P L A T	
2641	GCUUACCUGUGCGGGAUCCACCGGGAGGUAGUGCGCAGGCUUACAGCCGUCUUGCACCC	2700
	A Y L C G I H R E L V R R L T A V L L P	
2701	AACAUUCACACGCCUUUUUGACAUGUCGGCGGAGGACUUUGAUGCAAUCAUAGCAGAACAC	2760
	N I H T L F D M S A E D F D A I I A E H	
2761	UUCAAGCAAGGUGACCCGGUACUGGAGACGGAUACGCCUCCGUUCGACAAAAGCCAAGAC	2820
	F K Q G D P V L E T D I A S F D K S Q D	
2821	GACGCUAUGGCCGUUAACUGGCCUGAUGAUUUGGAGACCUGGGUGUGGACCAACCACUA	2880
	D A M A L T G L M I L E D L G V D Q P L	
2881	CUCGACUUGAUCGAGUGCGCCUUUUGAGAAAUAUCAUCCACCAUCUGCCCACGGUACC	2940
	L D L I E C A F G E I S S T H L P T G T	
2941	CGUUUCAAAUUCGGGGCGAUGAUGAAAUCGGAAUGUUCCUCACGCUCUUUGUCAACACA	3000
	R F K F G A M M K S G M F L T L F V N T	
3001	GUUCUGAAUGUCGUUAUCGCCAGCAGAGUAUUGGAGGAGCGGCCUUAAAACGUCCAAAUGU	3060
	V L N V V I A S R V L E E R L K T S K C	
3061	GCAGCAUUUAUCGGCGACGACAACAUACACCGAGUAGUAUCUGACAAAGAAAUGGUCA	3120
	A A F I G D D N I I H G V V S D K E M A	
3121	GAGAGGUGUGGCCACCUGGCUCACAAUGGAGGUUAAGAUCAUUGACGCAGUCAUCGGCGAG	3180
	E R C A T W L N M E V K I I D A V I G E	
3181	AGACCGCCUUACUUCUGCGGUGGUUCAUCUUGCAAGAUUCCGUACACAGCGUGU	3240
	R P P Y F C G G F I L Q D S V T S T A C	
3241	CGCGUGGCAGCCCUUGAAAAGGCUGUUUAAGUUGGUAAACCGCUCCCAGCGACGAC	3300
	R V A D P L K R L F K L G K P L P A D D	
3301	GAGCAAGACGAAGACAGAAGACGCGCUCUGCUAGAUGAAACAAAGGCUGGUUUAGAGUA	3360
	E Q D E D R R R A L L D E T K A W F R V	
3361	GGUUAACAGACACCUUAGCAGUGGCCUGGUACUCGGUAUGAGGUAGACAACAUACACA	3420
	G I T D T L A V A V A T R Y E V D N I T	
3421	CCUGUCCUGCUGGCAUUGAGAACUUUUGCCCAGAGCAAAAGAGCAUUUCAAGCCAUCAGA	3480
	P V L L A L R T F A Q S K R A F Q A I R	
3481	GGGGAAAAGCAUCUCUACGGUGGUCCUAAAAG	3516
	G E I K H L Y G G P K *	

Figure 4. Translated sequence of the nsP3-nsP4 region of the Girdwood strain of Sindbis isolated in South Africa. Conventions are the same as in Figure 2.

Figure 5. nsP3/nsP4 of Whataroa M78 (1962, New Zealand, mosquito pool)

1	GCGCCAUCGUACAAAUCAGGGAGAGGAAACAUCAUCGAAUGCACCAGAAGGCCGUG	60
	A P S Y K S R R G N I I E C T E E A V V	
61	AACGCUGCCAACGCACUAGGACGCCCGGAGAAGGGGUCUGCAAGGCAUUACAAGAAG	120
	N A A N A L G R P G E G V C K A I Y K K	
121	UGGCCGAACAGCUUCACCGGUUCCGCAACAGAAGUAGGGACUGCAAAAAUGACCACAAGC	180
	W P N S F T G S A T E V G T A K M T T S	
181	CUAGGCAAGAAAGUCAUACAUUGCCGUCGGACCGGAAUAAAAGAACGCACUCUGAAGAAGAA	240
	L G K K V I H A V G P D F K K H S E E E	
241	GCCCCUAAAACUGCUGCAGAAUGCCUACCGCAUCGCAGAUAAAUAUAAUGAGAACAC	300
	A L K L L Q N A Y H A I A D I I N E N N	
301	AUCAAAUCAGUGGCCAUUCCAUUGCUAUACUGGUAAUACGCUGCAGGGAAAGGACAGA	360
	I K S V A I P L L S T G I Y A A G K D R	
361	CUAGAGACUUCUUUGCACUGUUUGACCAAGCGAUGGACAGGACGGACGCCGACGUACG	420
	L E T S L H C L T T A M D R T D A D V T	
421	GUAUACUGCCUUGACAAGAAUGGCAGCGAGCGAAUUGACGCCAGUCCUAGAUUGAAAGAA	480
	V Y C L D K K W Q Q R I D A V L R L K E	
481	GAGGUAAACGGAGCUAAAAGACGACGACAUGGAAUUGAUGAGGAGCUGGUUUGGAUCCAC	540
	E V T E L K D D D M E I D E E L V W I H	
541	CCUGACAGCUGUUUGAAAGGACGUAAAGGCUUAGCACCCACCAAGGAAACUGUAUUCA	600
	P D S C L K G R K G F S T T K G K L Y S	
601	UACUUUCGAAGGAACUAAAUCACCCAGGCAGCGAAAGACAUUGCAGAAAUCAAUGUAUUG	660
	Y F E G T K F H Q A A K D M A E I N V L	
661	UUUCCAGACACCAUUGAGGCCUAACGAGCAAUCUGUAUGUAUAUCCUUGGAGAAAGCAUG	720
	F P D T I E A N E Q I C M Y I L G E S M	
721	GAAGCUAUCCCGAAAAAGCCCGUCGACUACAACCCUUCGUCAAGUCCGCCAAAACC	780
	E A I R E K C P V D Y N P S S S P P K T	
781	UUACCCUGCCUGUGCAUGUAUGCUAUGACACCUGAGAGGGUGCAUAGACUCAGAAC	840
	L P C L C M Y A M T P E R V H R L R S N	
841	AAUGUAAAGAAAUA CGGUUAUGCUCCUCGACUCCACUCCAAAACAUAAAUCAGAAC	900
	N V K E I T V C S S T P L P K H K I K N	
901	GUACAACGAAUCCAGUGUUCAAAAUCGUUUGUUUAUCCCGACACUCCAGCUUUGUA	960
	V Q R I Q C S K I V L F N P Q T P A F V	
961	CCUGCACGUAGGUCAUAGAAACCGAACCCAAAGAAACAGAACGAGCAUGCGGCUCAGCCG	1020
	P A R K F I E T E P K E T E D D A A Q P	
1021	GACCCGACACCGGUAGUGCAGGCAGUGUUUCGACCCGGUCCCACAACGUCAGCAAGAC	1080
	D P T P V V Q A S V S T P V P Q R Q Q D	
1081	CCGUUAGAGUUGAUAAAUCGCAGACUCUUUAACCGAAGUAAAACGACACCUCUGACGAC	1140
	P L E L I I S A D S L T E V N D T S D D	
1141	AUUUCCGACAUACCCUUUGACACAUUCGUUAUGCUAGUACUCCUCACUGAGCUCGGU	1200
	I S D I P F D T S V Y A S T S S L S S V	
1201	UUGGACUGCCACAAUGUAGUCGAGGUUCGAGGCCGAAAUUCAGUCGUCCCGCAGACUCCG	1260
	L D C H N V V E V E A E I H V V P Q T P	

Figure 5. nsP3/nsP4 of Whataroa M78 (1962, New Zealand, mosquito pool)

1261	GUGGCACCGCCGAGAAAGAAGAAGUAGCAGCUUUAGCGGCCUAUCAAGAGCAUCUAGC V A P P R K K K L A R L A A L S R A S S	1320
1321	AUUUCCUCCAUCGAAUCCAACCCACCAAUCACUUUUGGAUCAUUUGAGGAUGGGAGAAAUA I S S I E S N P P I T F G S F E D G E I	1380
1381	GACAACUUGCAGAAGAAGUGCACUUCAGAACCAUUUAUGUUCGGCUCGUUCGAACCAGGC D N L Q K K C T S E P F M F G S F E P G	1440
1441	GAAGUCAACAGCCUGAUAGAAACCAGGUCCGGAGCCACCACGUAGGGGGCGCAGACGUCGC E V N S L I E T R S E P P R R G R R R R	1500
1501	AACAAGAACCGACAGGAGAUUUGACUAACCGGGGUAGGUGGGUACAUUCUCGACGGAC N K N R Q E Y * L T G V G G Y I F S T D	1560
1561	ACUAAUGAAGGACACCUCCAGAAGAAAUCGGUACUCCAAAUAUGAUCUGGCAGUCACCAUU T N E G H L Q K K S V L Q N D L A V T I	1620
1621	UUAGAACGGAACAUUUGGAAAAAGUCCAUGCACCCGUGUACACGCUGAAAAAGAGGAG L E R N I L E K V H A P V Y N A E K E E	1680
1681	AUACUGAAAAUGAAGUACCAGAUGAUGGCCACCGAAACCAACAAGAGUCGGUACCAUCG I L K M K Y Q M M P T E T N K S R Y Q S	1740
1741	AGAAAAGUAGAAAUCAAAAGCAGUAACUACCCACGUCUAAUUAUCAGGACUGAACUU R K V E N Q K A V T T Q R L L S G L K L	1800
1801	UAUACAUUAUGAGCCUAACCAACCGGAGUGCUACAAAACCACAUAUCCGAGACCAUUGUAU Y T Y E P N Q P E C Y K T T Y P R P L Y	1860
1861	UCUAGUAGCAUACCAGUUAGUACGAUGCGCACAGUGGGCGUGCGAGUGCAUAAC S S S I P V S Y D S A Q V A V A V C N N	1920
1921	UACCUUGCAUGAAAACUAUCCGACUGUCGCACUUAACAGAUUACCGACGGAGUACGACGC Y L H E N Y P T V A S Y Q I T D E Y D A	1980
1981	UACCUAGACAUGGUGGAUGGCGCUGUCGUUGUCUGGACACAGCUACAUUUUGGUCCAGCU Y L D M V D G A V A C L D T A T F C P A	2040
2041	AAGCUCAGGAGCUUCCCGAAGAAGCAUGAAUUAAGACUCCCGAAAUCGCAGCGCUGUC K L R S F P K K H E Y K T P E I R S A V	2100
2101	CCCUCCGCCAUGCAGAACACACUACAGAAUGUACUCAUUGCCGCGACGAAACUGC P S A M Q N T L Q N V L I A A T K R N C	2160
2161	AACGUUACUCAGAUGCGAGAAUACCAACAUUGGAUUCAGCGACUUUAACGUGGAAUGC N V T Q M R E L P T L D S A T F N V E C	2220
2221	UUCAAAAAAUUUGCGUGUAUGACGAGUACUGGAGCGAAUUUCGUGACAACCCAUCA F K K F A C N D E Y W S E F R D K P I R	2280
2281	AUAACAAACCGAAUUCGUACCUUCGUACGUAGCGCGACUAAAAGGACCAAGGCAGCG I T T E F V T S Y V A R L K G P K A A A	2340
2341	UUGUUCGCAAAACCUAUACCUAGUUCCCUUGCAAGAACGUUCCUAUGGAUAGGUUGUC L F A K T H N L V P L Q E V P M D R F V	2400
2401	AUGGACAUGAAGAGGGACGUAAAAGUCACACCGGAACAAACACACAGAAGAGAGACCA M D M K R D V K V T P G T K H T E E R P	2460
2461	AAAGUCCAAGUCAUCCAGGCCUGAGCCGUAGCUACCGCAUACUUUGCGGAAUCCAC K V Q V I Q A A E P L A T A Y L C G I H	2520

Figure 5. nsP3/nsP4 of Whataroa M78 (1962, New Zealand, mosquito pool)

2521	CGAGAACUGGUUAGGAGGCUGACUGCUGUACUACUUCGAACAUUCACACCCUGUUUCGAU R E L V R R L T A V L L P N I H T L F D	2580
2581	AUGUCGGCCGAAGAUUUUGACGCUAUCAUAGCUGAACAUUCAACUAUGGGACCCUGUC M S A E D F D A I I A E H F N Y G D P V	2640
2641	UUAGAAACCGACAUCGCGUUCGACAAAAGUCAGGACGACGCCAUGGCCUGACCGGC L E T D I A S F D K S Q D D A M A L T G	2700
2701	CUGAUGAUCCUUGAAGACUUGGGUGUCGACCAGCCCCUUUAGACCUUAUUGAAUGUGCG L M I L E D L G V D Q P L L D L I E C A	2760
2761	UUCGGCGAAACUCCUCGACGCAUCUCCGACAGGUACGAGAUUCAAAAUUUGGAUCGAUG F G E I S S T H L P T G T R F K F G S M	2820
2821	AUGAAAUCUGGAUGUUCCUCACCCUGUUUGUCAACACACUGUGCUGAAUGUUGUAAUCGCC M K S G M F L T L F V N T V L N V V I A	2880
2881	AGCAGGGGUCCUAGAGCAUAGACUGAAAGAGUCGCCGAUGC CGCAAUCAUCGGUGAUGAC S R V L E H R L K E S R C A A F I G D D	2940
2941	AACAUAAAUCACGGCGUAGUGUCUGACAAGGAAAUUGGCAGAAAGAUGCGCUACCUUGGU N I I H G V V S D K E M A E R C A T W L	3000
3001	AACAUUGGAAGUGAAGAUCAUCGACGCCGUCAUAGGCAUCAGACCUCCAUAUUUUGUGGU N M E V K I I D A V I G I R P P Y F C G	3060
3061	GGAUUCAUCCUUCAAGAUGAGACGACAUUAACCACAUGUCGCGUCGCCGAUCCGCUUAAG G F I L Q D E T T L T T C R V A D P L K	3120
3121	AGGCUCUUAAAACUAGGUAAACCACUACCCGGAGGACACGCAAGAUGAAGACAGAAGA R L F K L G K P L P A E D T Q D E D R R	3180
3181	CGUGCCCUUAUGGACGAAACCAAAGCAUGGUUCCGGGUAGGAAUUAGGAACACUCUCGCA R A L M D E T K A W F R V G I R N T L A	3240
3241	GUUGCCGUAUCGACCAGGUACGAGGUAGAAGAUUUACCCGUUCUAUACGCCGUAGA V A V S T R Y E V E D I T P V L Y A L R	3300
3301	ACAUUCGCUAAAGCAAAAAGCCUUCAGACUAUACGAGGAGAAAUAAGACAGCUCUAC T F A Q S K K A F Q T I R G E I R Q L Y	3360
3361	GGCGGUCCUAAAAG 3375 G G P K *	

Figure 5. Translated sequence of the nsP3-nsP4 regions of Whataroa virus, isolated in New Zealand. It is clear from this sequence that Whataroa virus is closely related to Sindbis virus.

**Amino acid differences in N-terminal half of nsP3 (%)**

	Girdwood	Ockelbo	A1036	MRM18520	Whataroa
AR339	1.8	1.8	10.2	9.5	15.4
Girdwood		0.3	8.9	8.6	15.4
Ockelbo			9.2	8.9	15.4
A1036				1.5	16.0
MRM18520					16.3

**Amino acid differences in nsP4 (%)**

	Girdwood	Ockelbo	A1036	MRM18520	Whataroa
AR339	1.3	1.6	7.7	7.7	11.5
Girdwood		0.3	7.4	7.4	12.1
Ockelbo			7.7	7.7	12.4
A1036				1.6	12.4
MRM18520					11.8

**Figure 6.** Percent amino acid differences between the different isolates of Sindbis virus in two regions of the nonstructural proteins.

development of techniques for construction of such a library. The details of the methods developed are presented in the Methods section and required a careful attention to detail in order to obtain a random library. With the automated DNA sequencer, 24 DNA samples can be analyzed at one time and each sample can be read automatically to more than 400 nucleotides. Thus about 10 kb of sequence is obtained from a single run. To obtain the complete sequence of a viral RNA requires over-sequencing of the genome because of compression artifacts and occasional misreading by the machines and a slightly nonrandom distribution of the sequences obtained. The procedure developed as the most efficient is to over-sequence about three-fold, that is to obtain about 30 kb of sequence for the 12 kb RNA, align this sequence using computer programs and using the homology between different alphaviruses, and then to fill in any gaps that might still exist by designing PCR primers that can be used to obtain double-stranded cDNA for the missing segments and sequence this DNA manually. Fig. 7 illustrates the distribution of cDNA clones obtained using the technology developed for Whateroa virus and shows the random nature of the clones obtained. Fig. 8 illustrates sequence output from the Applied Biosystems sequenator for one clone (the original output is in four colors, a different color for each of the four nucleotides, which aids in interpreting the data). This sequence is automatically recorded in a computer file. Fig. 9 shows the DNA sequence obtained from this clone of Whateroa virus and compares the sequence to that of Sindbis virus. The technology is highly suitable to obtaining large amounts of sequence from alphaviruses and makes it conceptually feasible to examine a large number of different alphaviruses or of strains of one alphavirus isolated from different locations of the world in order to examine the relationships of the viruses to one another. The sequence of the nsP3-nsP4 region of Whataroa virus obtained by this method was shown in Fig. 5.

### **Mapping of a Neutralizing Antibody-Binding Site in Glycoprotein E2**

A λgt11 library containing randomly generated 100-300 base pair Sindbis cDNA inserts in the lacZ gene was tested for reactivity with 6 mAbs, using <sup>125</sup>I-protein G to detect the presence of mAb (all were IgGs) bound to immunoreactive phage clones on nitrocellulose filters. Four

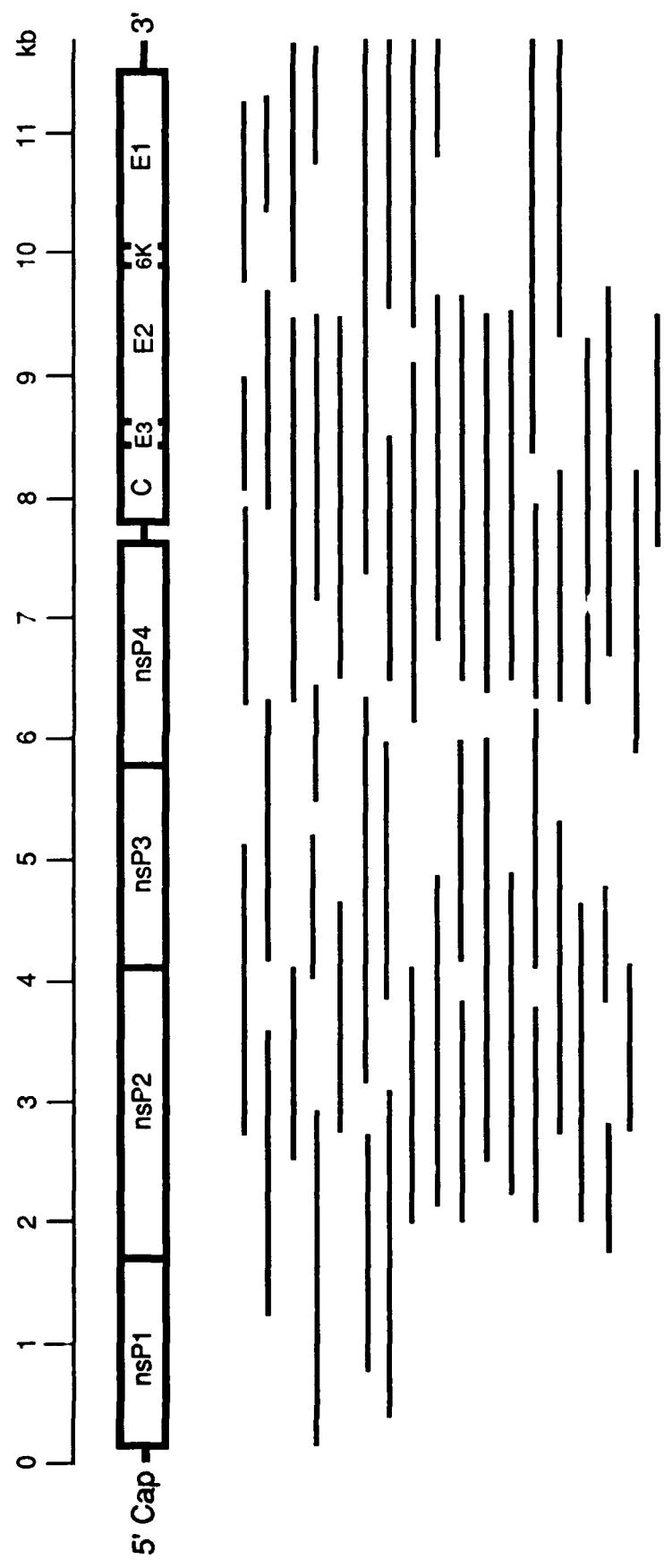
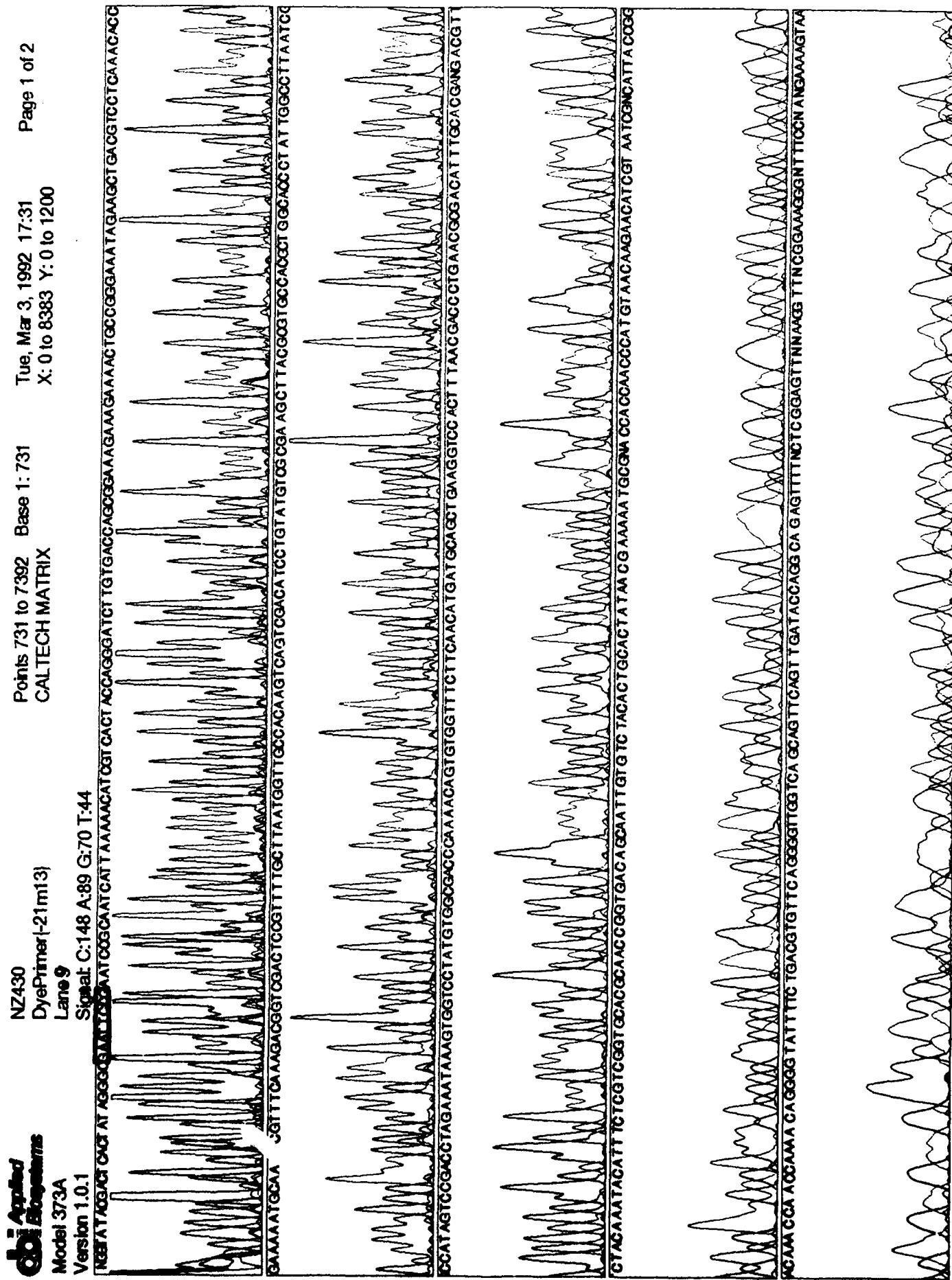


Figure 7. Map of the random cDNA clones of Whataroa virus which have been characterized. The top line is a scale in kilobases, and below that is shown a simple map of the alphavirus genome. Each line below represents a cloned insert which has been mapped by aligning its sequence with that of Sindbis virus.



**Figure 8.** Automated sequence analysis of clone NZ430 of Whataaroa virus.

2200 CGGTCCCGTACAAGGTCGAACAAATAGGAGTGTAGGCACACCGGGTCG 2249  
 1 ..... NGGTATA~~CGACTCACTATAGGGC~~GAAT 27  
 2250 GGCAAGTCAGCTATTATCAAGTCACGTACGGCACGAGATCTTGTAC 2299  
 28 TCCGAATCCGCAATCATTAAAAACATCGTCACTACCAGGGATCTTGTGAC 77  
 2300 CAGCGGAAAGAAAGAAAATTGTCGCCAATTGAGGCCGACGTGCTAAGAC 2349  
 78 CAGCGGAAAGAAAGAAA~~ACTGCCGGAAATAGAAGCTGACGT~~CCTCAAAC 127  
 2350 TGAGGGGTATGCAGATTACGTCAAGACAGTAGATTGGTTATGCTCAAC 2399  
 128 ACCGAAAAATGCAAATCGTTCAAAGACGGTCACTCCGTTTGCTTAAT 177  
 2400 GGATGCCACAAAGCCGTAGAAGTGTGTACGTTGACGAAGCGTCGCGTG 2449  
 178 GGTTGCCACAAGTCAAGTCAGTCACATCCTGTATGTCG.CGAAGCTTACGCGTG 226  
 2450 CCACGCAGGAGCACTACTTGCCTGATTGCTATCGTCAGGCCCGCAAGA 2499  
 227 CCACGCTGGCACCCATTGGCCTTAATGCCATAGTCCGACCTAGAAATA 276  
 2500 AGGTAGTACTATGCGGAGACCCCATGCAATGCGGATTCTCAACATGATG 2549  
 277 AAGTGGTCCTATGTGGCGACCCAAA~~ACAGTGTGGTTCTCAACATGATG~~ 326  
 2550 CAACTAAAGGTACATTCAATCACCC~~TGAAAAAGACATATGCACCAAGAC~~ 2599  
 327 CAGCTGAAGGTCCACTTAACGACCC~~TGAACGCGACATTG~~CACGANGAC 376  
 2600 ATTCTACAAGTATATCTCCCGCGTTGCACACAGCCAGTTACAGCTATTG 2649  
 377 GTTCTACAATACATTCTCGTCGGTGCACGCAACC~~GGT~~GACAGCAATTG 426  
 2650 TATCGACACTGCATTACGATGGAAAGATGAAAACCACGAACCCGTGCAAG 2699  
 427 TGTCTACACTGC~~ACTA.TAACGAAAATGCGNACCACCAACCCATG~~TAAC 475  
 2700 AAGAACATTGAAATGATATTACAGGGGCCACAAAGCCGAAGCCAGGGGA 2749  
 476 AAGAACATCGTAATGNCAATTACCGGACAAACCAACCAAA~~ACAGGGT~~TAT 525  
 2750 TATC.....ATCCTGACATGTTCCGCGGGTGGGTTAAGCAATTGCAAA 2793  
 526 TTTCTGACGTGTT~~CAGGGTTGGT~~CAGCAGTTGATACCAGGCAGA 575  
 2794 TCGACTATCCGGACATGAAGTAATGACAGCCGCGCCTCACAAGGGCTA 2843  
 576 GTTTNCTCGGAGTTNNAAAGGTNC..... 600

Figure 9. Comparison of the sequence of clone NZ430 of Whataroa virus (from data in Figure 8) with the sequence of Sindbis virus. Vertical lines between nucleotides highlight identity; vertical dots show ambiguities. Underlined sequence is that of the EcoRI linker used to construct the clone; sequence upstream of this is vector.

positive phage clones were identified when mAb 23 was used to screen  $10^6$  plaques, designated  $\lambda 23a$ ,  $\lambda 23b$ ,  $\lambda 23c$  and  $\lambda 23d$ . Results with two of these clones are illustrated in Fig. 10; also shown is a control in which a nonreactive region of E2 is present as an insert in the  $\lambda gt11$  clone. These four phages were plaque purified and DNA prepared from each (Young and Davis, 1983). The inserts were removed with *EcoRI*, subcloned into vector M13mp18, and sequenced by the dideoxy chain termination procedure (Sanger et al., 1977). The four inserts contained overlapping sequences from the central region of glycoprotein E2 (Fig. 11). The insert in  $\lambda 23a$  comprised E2 residues 155-258, that in  $\lambda 23b$  comprised residues 173-251, that in  $\lambda 23c$  145-223, and that in  $\lambda 23d$  169-220. Thus the domain from residues 173 to 220 is present in all four inserts, and the neutralizing epitope recognized by mAb 23 must lie within this region. It is of note that this overlap region is 2-3 fold larger than the 15-22 amino acid residues found to contact antibody in epitopes defined by X-ray diffraction analysis (Laver et al., 1990), and it is conceivable that the epitope could be formed by a folded structure with contributions from residues throughout this region.

We also attempted to identify fusion proteins immunoreactive with four other E2-specific neutralizing mAbs, namely mAbs 18, 50, 51, and 49, as well as fusion proteins immunoreactive with mAb 33, specific for glycoprotein E1. In each case  $10^6$  plaques were screened. No positive plaques could be identified with any of these antibodies. We concluded that these antibodies probably react with conformational epitopes not present in the  $\lambda gt11$  library, either because these epitopes are discontinuous or consist of conformations not assumed by the fusion proteins.

### Conclusions

**Rapid Sequencing of Virus RNAs.** High throughput automated DNA sequencing is ideally suited to obtaining large amounts of sequence data for strains of alphaviruses or for other viruses. The methods that we have developed can be used for any RNA virus and are suitable to

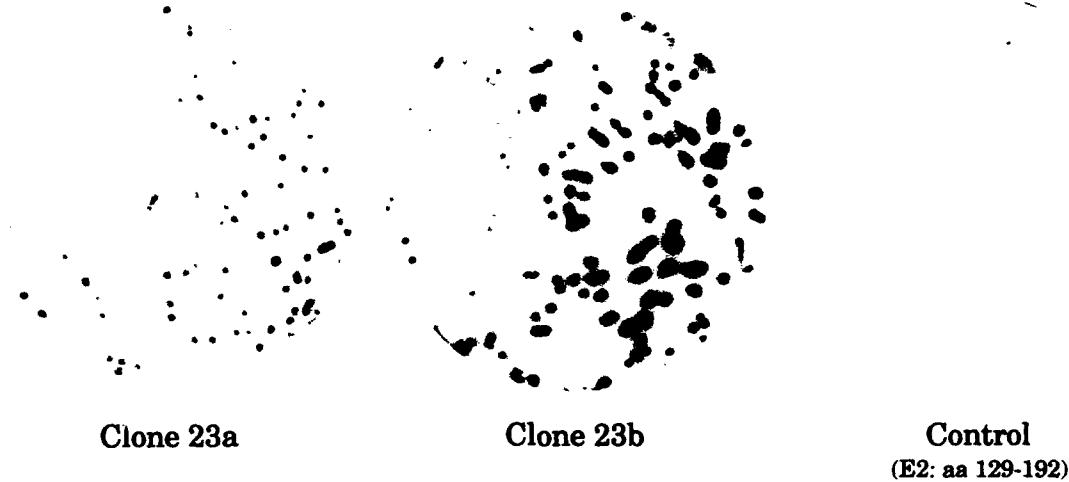


Figure 10. Reactivities of phage clones  $\lambda$ 23a and  $\lambda$ 23b with MAbs 23. Immunoreactive phage plaques were picked and rescreened until a uniformly reactive population was obtained. Illustrated are the final populations for two reactive clones and a nonreactive clone expressing amino acids 129 - 192 of E2 (control). Phage stocks were plated on *E. coli* Y1090 in the presence of the inducer and the plaques transferred to nitrocellulose. Filters were incubated with MAbs 23 followed by  $^{125}\text{I}$  conjugated protein G and autoradiographed. Comparison of the autoradiogram with the pattern of plaques on the petri plate showed that all of the  $\lambda$ 23a and  $\lambda$ 23b plaques reacted with the antibody.

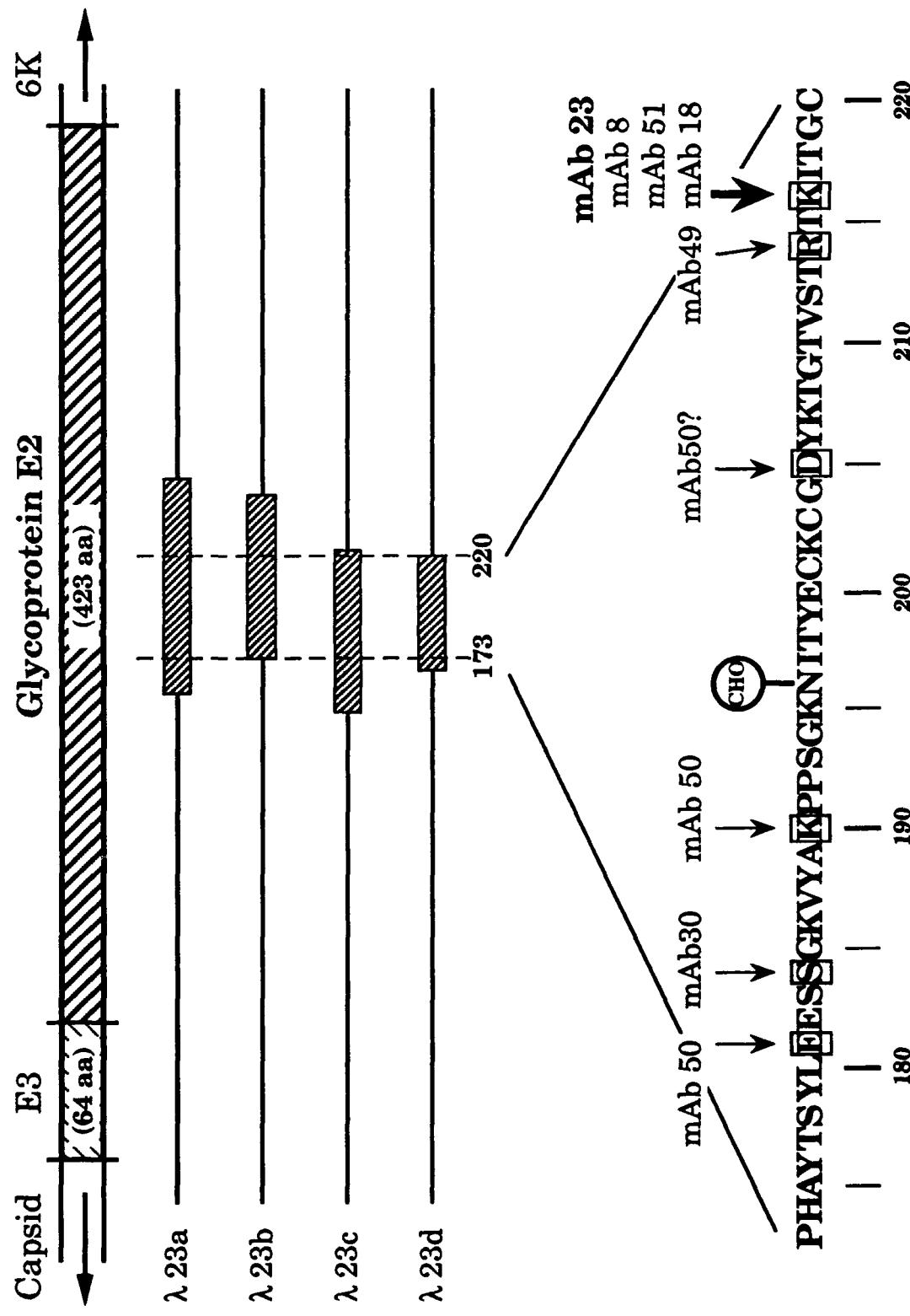


Figure 11. Schematic representation of an antigenically important domain of Sindbis virus glycoprotein E2. The relative locations of the inserts in four  $\lambda$ gt11 clones reactive with MAb 23 are mapped. The overlap region in these four clones between residues 173 and 220 of E2 is expanded below, with a number of key features indicated. Residues altered in variants resistant to MAbs are boxed and a carbohydrate attachment site is indicated with a stalked symbol (CHO).

obtaining sequence information for different viruses belonging to a virus group or to obtaining sequence for strains of the same virus isolated from different geographic regions. This makes it feasible to examine a large number of isolates and therefore to determine the relationships among a group of viruses or to search for emergent viruses that differ in certain fundamental ways from other members of the group. The sequences presented in this report are an example of what it is possible to do. These sequences examine the relationships among a number of different geographic isolates of Sindbis-like viruses.

**An Antibody Binding Domain in E2.** The  $\lambda$ gt11 system provides a rapid, specific, and sensitive strategy for the physical mapping on large viral genomes of the genes encoding proteins for which antibody reagents are available. We used small Sindbis virus genomic inserts in an attempt to define the immunoreactive domain of the protein more precisely. The limitation of the  $\lambda$ gt11 system is the fact that these protein domains are expressed as part of a fusion protein and thus may not fold in the same way as the native protein, and only antibodies that interact with contiguous linear domains of the proteins of interest may be reactive with phage plaques.

From the sequence of the inserts in the four clones immunoreactive with mAb 23, it is clear that this antibody can react with a single continuous region of the Sindbis glycoprotein E2, and that the neutralization epitope must lie within the 48 residues between amino acids 173 and 220. This result is consistent with the results from mapping of antibody escape variants resistant to mAb 23 (Fig. 10). Sequencing of 3 independent variants resistant to mAb 23 and of 2 independent revertants selected to be sensitive again to mAb 23, as well as of other variants, has shown that residue 216 is important for reactivity with mAb 23 (Strauss et al., 1991). Virus with Lys-216 were fully sensitive to mAb 23, virus with Val-216 or Ile-216 demonstrated a reduced sensitivity to mAb 23, and virus with Glu-216 were resistant to mAb 23. From the results obtained here, it appears likely therefore that residue 216 interacts directly with mAb 23.

Although the remaining antibodies tested failed to react with the  $\lambda$ gt11 library, presumably because they react with conformational epitopes not present in the library, it seems likely that the

E2-specific mAbs 50, 51, 49, and 18 also bind to epitopes at least partially encompassed within this same domain. Variants selected to be resistant to these mAbs were all found to have amino acid changes responsible for the escape from neutralization within the domain from residues 181 to 216 (Fig. 1) (Strauss et al., 1991). Furthermore, mAb 23 and these mAbs all react with closely spaced or overlapping epitopes as defined by competition assays or by the pattern of cross reactivity of different variants resistant to the various antibodies (Davis et al., 1987; Schmaljohn et al., 1983; Strauss et al., 1991). The results are all consistent with the hypothesis that the E2 domain between 173 and 220 forms a major antibody binding region important for neutralization of virus infectivity. This domain is illustrated in Fig. 11 with the locations of antibody escape variants shown and the region selected by mAb 23 indicated. This domain is hydrophilic, containing 25% charged residues, and has a glycosylation site at Asn-196, and thus is almost certainly exposed on the surface of the glycoprotein spike (Strauss and Strauss, 1986).

We have previously found that an antiidiotypic antibody to mAb 23, as well as antiidiotypic antibodies to mAbs 49 and 50, function as antireceptor antibodies in chicken cells (Wang et al., 1991). This suggests that the E2 domain defined by the fusion protein reactive with mAb 23 and by the antibody escape variants might form part of the antireceptor on the virus spike that binds to the cellular receptor. This hypothesis is supported by the observation that two strains of Sindbis virus that differ only in having Gly or Arg at residue 172 of E2 differ in their ability to bind to neuroblastoma cells in culture (Tucker and Griffin, 1991) and differ in their neurovirulence for mice (Lustig et al., 1988).

These results make clear that the region of E2 between residues 170 and 220 contains a number of dominant epitopes, and that this region is a key region for the development of vaccines.

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